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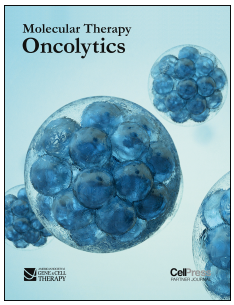

Oncograms visualize factors influencing long term survival of cancer patients treated with adenoviral oncolytic immunotherapy

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PII: S2372-7705(18)30010-X

DOI: 10.1016/j.omto.2018.04.003

Reference: OMTO 49

To appear in: *Molecular Therapy: Oncolytics*

Received Date: 6 March 2018

Accepted Date: 17 April 2018

Please cite this article as: Hemminki O, Oksanen M, Taipale K, Liikanen I, Koski A, Joensuu T, Kanerva A, Hemminki A, Oncograms visualize factors influencing long term survival of cancer patients treated with adenoviral oncolytic immunotherapy, *Molecular Therapy: Oncolytics* (2018), doi: 10.1016/j.omto.2018.04.003.

Baseline patient features

		Best survivors		All ATAP	
Sex		N	%	N	%
	Male	8	27 %	167	42 %
	Female	22	73 %	123	58 %
Age					
	Median	58		58	
	Mean	54		56	
	Range	6-78		3-85	
WHO performance status		N	%	N	%
	0	8	27 %	29	10 %
	1	18	60 %	129	44 %
	2	4	13 %	114	39 %
	3	0	0 %	18	6 %
	Median	1		1	
	Mean	0,9		1,4	
Cancer type		N	%	N	%
	Breast	4	13 %	35	12 %
	Cervical	0	0 %	6	2 %
	Colorectal	1	3 %	49	17 %
	Hepato/cholangio	1	3 %	8	3 %
	Head and neck/Thyroid	3	10 %	12	4 %
	Gastric	0	0 %	11	4 %
	Lung	4	13 %	22	8 %
	Melanoma	1	3 %	15	5 %
	Meso/sarcoma	6	20 %	36	12 %
	Neuroend/-blast	1	3 %	5	2 %
	Ovarian	7	23 %	39	13 %
	Pancreatic	0	0 %	30	10 %
	Prostate	2	7 %	14	5 %
	Urinary tract	0	0 %	8	3 %
Previous treatments		N	%	N	%
	Surgery	22	70 %	195	67 %
	Radiotherapy	14	47 %	142	49 %
	Chemotherapy	29	93 %	287	99 %
	Median chemo regimens	3		4	
	Mean chemo regimens	4,1		4,2	
	Range of chemo regimens	0-14		0-15	

Patient treatments, responses and survival

		Best survivors		All ATAP	
		30	100 %	290	100 %
Viral treatments		N	%	N	%
	1-3	15	50 %	240	83 %
	4-8	10	33 %	43	15 %
	8-18	5	17 %	7	2 %
	mean	5,6		2,8	
	median	4		3	
Serial treatment *		7	23 %	154	53 %
Low-dose cyclophoshamide **		26	87 %	223	77 %
First imaging response					
	CR/CMR	7	23 %	9	3 %
	PR/PMD	3	10 %	5	2 %
	MR/MMR	3	10 %	16	6 %
	SD/SMD	11	37 %	40	14 %
	PD/PMD	5	17 %	106	37 %
	NA	1	3 %	114	39 %
Best imaging response					
	CR/CMR	7	23 %	9	3 %
	PR/PMD	3	10 %	5	2 %
	MR/MMR	4	13 %	18	6 %
	SD/SMD	12	40 %	44	15 %
	PD/PMD	3	10 %	100	34 %
	NA	1	3 %	114	39 %
Best marker response***					
	CR	1	3 %	3	1 %
	PR	0	0 %	12	4 %
	MR	5	17 %	26	9 %
	SD	0	0 %	24	8 %
	PD	5	17 %	65	22 %
	NA	19	63 %	160	55 %
OS mean		1186		265	
OS median		921		132	

*Patient received at least one “serial treatment” (3 treatments during a 10 week period , see patients and methods)
** low dose cyclophosphamide was used to reduce regulatory T-cells (see patients and methods)
*** tumor marker molecules measured from blood to evaluate treatment effects
CR/CMR=Complete response/complete metabolite response
PR/PMR=Partial response/partial metabolite response, >30% reduction

MR/MMR=Minor response/minor metabolite response, 12-29% reduction
SD/SMD=Stable disease/stable metabolite disease
PD/PMR=Progressive disease/Progressive metabolite disease >30% increase
NA = not assessable

Oncograms visualize factors influencing long term survival of cancer patients treated with adenoviral oncolytic immunotherapy

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Keywords, 5 to 10 keywords.

oncolytic adenovirus; oncolytic virotherapy; immunotherapy; cancer; immunostimulation; anti-cancer; adenovirus

Funding details.

This study was supported by The Finnish Medical Foundation, Jane and Aatos Erkko Foundation, HUCH Research Funds (EVO), Sigrid Juselius Foundation, Finnish Cancer Organizations and University of Helsinki.

Disclosure statement.

A.H. and O.H. are shareholders in Targovax ASA and TILT Biotherapeutics Ltd. A.H. is employee in

TILT Biotherapeutics Ltd.

Abstract

The first FDA and EMA approved oncolytic virus has been available since 2015. However, there are no markers available that would predict benefit for the individual patient. During 2007-2012 we treated 290 patients with advanced chemotherapy refractory cancers, using 10 different oncolytic adenoviruses. Treatments were given in a FIMEA regulated individualized patient treatment program (the Advanced Therapy Access Program ATAP), which required long term follow-up of patients, which is presented here. Focusing on the longest surviving patients, some key clinical and biological features are presented as "oncograms". Some key attributes that could be captured in the oncogram are suggested to predict treatment response and survival after oncolytic adenovirus treatment. The oncogram includes immunological laboratory parameters assessed in peripheral blood (leukocytes, neutrophil-to-lymphocyte ratio, IL-8, HMGB1, anti-viral neutralizing antibody status), features of the patient (gender, performance status), tumor features (histological tumor type, tumor load, region of metastases) and oncolytic virus specific features (arming of the virus). The retrospective approach used here facilitates verification in a prospective controlled trial setting. To our knowledge the oncogram is the first holistic attempt to identify the patients most likely to benefit from adenoviral oncolytic virotherapy.

Introduction

Cancer immunotherapy has provided several exciting breakthroughs during the past few years. Our growing understanding of molecular biology, immunology and cancer genetics has led to

several new treatments able to generate durable responses. For most types of advanced cancers this is a new situation since surgery, chemotherapy, radiation, kinase inhibitors and hormonal therapies are usually not curative when the patient has metastatic disease.

Checkpoint inhibitors have shown efficacy in a variety of tumors and approval is likely for several new indications in addition to the half dozen already approved[1-3]. Also different cell based therapies have shown promising results over the past few decades and two products have been approved[4, 5]. Oncolytic viruses have progressed steadily in trials and the first FDA and EMA approvals were granted in 2015[6], with further viruses likely to be approved later. Interleukin-2 and interferon alpha have been used with variable enthusiasm for a few decades and some patients show durable long term responses[7]. Probably the most routine use of immunotherapy has been the Bacillus Calmette-Guérin (BCG) for superficial bladder cancer[8]. With all this excitement it can be forgotten that each of these immunotherapies only work in a subgroup of patients. For example, when used as single agents, FDA approved checkpoint inhibitors only provide responses in 10-50% of patients, depending on tumor type[1-3].

It would be of key relevance to identify the patients most likely to benefit from each approach. Human suffering could be reduced and monetary resources saved if patients would be directly treated with the most effective drug or combination, especially if long term efficacy results.

Emerging evidence suggests that the immune status of tumors varies[9]. Tumors can be grouped roughly into “hot”, “immunologically excluded” and “cold” tumors[3, 10, 11]. The latter two types are often combined, resulting in just two groups: “hot” and “cold”. A typical “hot” tumor has a high mutational load, in particular featuring neoantigens and subsequently ample CD8+ T-cells recognizing said mutations. In theory such T-cells should result in tumor destruction but obviously this had not happened if the patient was diagnosed with cancer. Since any immune reaction

results in an immunosuppressive counter-reaction, it is logical that “hot” tumors typically display programmed death ligand-1 (PD-L1) expression, which is one of the factors associated with T-cell anergy and survival of tumor cells. In such “hot” tumors checkpoint inhibitors that block the PD-1/PD-L1 interaction are known to result in high response rates[12].

These developments underline the utility of understanding the underlying molecular mechanisms for optimal patient selection. This is employed in lung cancer for example, where some anti-PD-1 drugs are only approved for PD-L1 positive tumors[3].

In “cold” tumors the mutational load of the tumor is generally lower and the tumor tissue lacks cells of the adaptive immune system which may indicate that the immune system has been unable to recognize the tumor. Thus also T-cell activating checkpoint inhibitors have generally poor efficacy[13]. Emerging data suggest that agents such as oncolytic viruses are able to cause inflammation, tumor cell destruction and activation of the immune system against these tumors[10, 11, 13-16]. In essence, oncolytic viruses may be able to convert “cold” into “hot” tumor, making them uniquely attractive in this subgroup of patients[11, 17].

During 2007-2012 290 patients were treated with oncolytic adenoviruses in an Advanced Therapy Access Program (ATAP)[6]. Altogether ten different viruses were used in 821 individualized treatments[18-23]. The adenoviruses used were engineered so that they could replicate only in tumor cells. Most of these viruses were based on serotype 5, but some had the fiber knob of serotype 3[11, 19] (to enhance tumor transduction) and one virus was fully serotype 3 based[24, 25]. Adenovirus infection *per se* induces immunogenic cell death[26] but to further activate the immune system some viruses were armed with immunostimulatory molecules GM-CSF[11, 20] or CD40L[27]. In many patients imaging or tumor marker analysis suggested efficacy but some patients seemed not to

benefit from the treatments. Using retrospective analysis we have previously been able to recognize several factors that seemed to correlate with good responses and survival[14, 18, 25, 27-29].

Here we have attempted to analyze and refine the clinical and biological information gleaned from the patient treatment program. Inspired by the cancer immunogram published by Blank et al [9], we developed an oncolytic virus specific "oncogram" to present the key predictive and prognostic factors in a compact and visual way using actual patients as examples. We have not seen a similar patient-by-patient approach for oncolytic viruses or other immunotherapeutics – the model published by Blank et al was largely theoretical [9]. We believe the oncogram is a practically usable tool for identifying cancer patients most likely to benefit from oncolytic adenovirus treatment, and could apply also to other viruses although this remains to be studied.

Patients and Methods

In the advanced therapy access program (ATAP) patients were treated in an individualized patient by patient basis - not according to a preplanned study protocol. Different oncolytic adenoviruses were used to treat various types of solid tumors. Treatments are described in more detail elsewhere [6, 11, 24, 25 14, 18, 27]. Treatments took place in Docrates Hospital, Helsinki, Finland. In most cases virus was injected directly in to the tumor by a radiologist, but also intravenous and intraperitoneal treatment was utilized. As described previously [6, 11, 24, 25 14, 18, 27], treatments were well tolerated. In general, tumor pain, flu-like symptoms, fever and fatigue resulted from treatment.

In the present evaluation data from the thirty patients with the longest survival was included. These patients were compared to all patients in the ATAP and to the worst surviving patients. The worst surviving controls were adjusted by cancer type so that whenever possible the same amount

of patients per cancer type were taken as controls; for example the four best surviving breast cancer patients were compared to the four breast cancer patients who survived the least.

Oncograms include eleven predictive or prognostic variables with patients treated with oncolytic immunotherapy [14]. Individual patient oncograms are designed so that good variables (recorded before first oncolytic virus treatment) are present at the outer ring, these include: 1) female gender 2) WHO 0-1 3) cancers other than melanoma, colorectal, pancreatic, hepatocellular or cholangio carcinoma 4) low tumor load 5) peritoneal metastases without liver metastases 6) low neutrophil to lymphocyte ratio (low neutrophils and/or high lymphocytes) 7) low leucocyte value 8) low IL-8 9) low HMGB1[27] 10) First treatments with GM-CSF or CD40L armed virus 11) no anti-viral neutralizing antibodies[14]. Inner ring values include poor prognostic variables. Variables that were not available are marked in the middle ring and the label was removed from the oncogram. Patients that had liver metastases (poor prognostic marker)[14] and peritoneal metastasis (good prognostic marker) or no metastasis were also marked at the middle ring. In figure 4 a) where oncogram averages are presented by tumor type we took into account also values that were not available by using a value of 0.5 while in panel b) the not-available values were left out. This was due to low number of variables present in panel a) and thus single available variables would have distorted the average oncogram considerably. Especially laboratory analysis were not available for some patients before treatments. In statistical analyses the not-available values were naturally not taken into account.

The retrospective analysis of these patients was approved by the HUS Operative Ethics Committee and the treatments were in accordance with the Declaration of Helsinki. Survival information was obtained from the Finnish Population Registry.

Statistical analyses were performed using Student's t-test and Fisher´s exact test in the case of figure 4 (as suggested by a statistican). Two-tailed test was used and p values of <0.05 were considered significant.

Serial oncolytic viral treatment where three injections of virus during a ten week period was given to part of the patients before evaluating treatment efficacy [30]. Low dose cyclophosphamide was used to reduce regulatory T-cells[31].

Results

To date 5-10 year follow up of ATAP[6] patients is possible. All patients had advanced solid tumors, and had gone through routine evidence-based treatments before entering ATAP (Supplementary Table 1). Most patients were heavily pre-treated with a median of 4 prior lines of medical therapies (Table 1). In this study we focused on the thirty longest surviving patients, their tumor type matched controls (with short survival) and compared them with the overall ATAP population (Figure 1). The median survival of these long term survivors (N=30) was 921 days, while the median survival in the general ATAP population (N=290) was 132 days and the worst surviving controls (N=26) had a survival of only 51 days underlining the advanced disease status of typical ATAP patients.

Slightly more patients participating in ATAP were female (58%, Table 1). In contrast, 73% of the long term survivors were women (p=0.001). The general performance status of the long term survivors was also better (87% WHO/ECOG 0-1) compared to all ATAP patients (54% WHO/ECOG 0-1, p<0.001). Ovarian cancer, lung cancer, mesothelioma and sarcoma patients seemed to be common in the best survivors while only 1 of the 79 patients with colorectal or pancreatic cancer patients contributed to this group. Age and number of previous treatments were similar between the groups.

Long term survivors received a higher number of viral treatments (50% had received 4-18 treatments, Table 2) compared to the overall ATAP population (19% received 4-18 treatments, p=0.002). This doesn't necessarily indicate causality since it is logical that if the patient was alive they might want to continue therapy. Of the best survivors 23% presented a complete response or a continuing complete response evaluated by computed tomography (CT) or by positron emission tomography–computed tomography (PET-CT) while in all ATAP patients this was seen only in 3% (p<0.001). Similar findings were recorded with patients who had evaluable tumor markers before virus treatments. Over half (6 out of 11) of the evaluable best survivors showed a marker response compared to less than one third (41 out of 130) of all evaluable ATAP patients (p=0.12). The first imaging response was typically also the best response. In ATAP overall, 53% of patients received a more intensive "serial treatment" (three treatments within 10 weeks)[30]. Interestingly, only 23% of the best survivors had received this (p=0.002). Again, there might not be causality. Instead, it could be that if the first injection immediately shrinked tumors or the tumors were small/technically demanding to inject, it might have been difficult to continue with intratumoral injections.

Oncograms of the best surviving 30 patients are shown in figure 2 and the oncograms of the worst surviving control patients are shown in supplementary figure 1. Each oncogram consists of 11 variables that have been considered important in previous publications from this ATAP cohort [14, 18, 25, 27-29]. These significant factors that seem to affect survival of the patients are summarized in supplementary table 2. Favorable variants (see patients and methods for details) are placed on the outer ring and non-favorable variants on the inner ring, similarly as in the previously published immunogram[9]. If the value was not known the label has been removed and the line was left on the

middle ring. In the case of metastases, liver metastases have been reported to indicate poor prognosis and were placed on the inner ring while peritoneal metastases have been reported favorable[14] and were thus marked on the outer ring. If metastases were in other organs, there were no metastases or metastases were both peritoneal and hepatic, this was marked on the middle ring.

With this system where favorable factors are always on the outer ring and unfavorable on the inner ring, oncograms with a large surface area indicate patients with many favorable factors in the context of oncolytic adenovirus treatments. In contrast, oncograms with small surface area propose the opposite.

In the best surviving thirty patients there were seven ovarian cancer patients (patient code starting with O, figure 2). If we compare these to the worst surviving ovarian cancer patients (Supplementary Fig 1) even the patient by patient oncograms appear different. This exemplifies how the oncogram can be used for clinical decision-making; perhaps ovarian cancer patients with a small area oncogram should have received another type of therapy. Obviously, this remains to be prospectively studied. The oncogram can also be used to generate biological hypotheses. For example, it was interesting to note that many of the long surviving ovarian cancer patients had peritoneal metastasis while the short surviving patients seemed to have unfavorable neutrophil to lymphocyte ratio. Perhaps partially explaining this, it has been speculated previously that the peritoneal cavity can be considered an immunological organ[30, 32], while the neutrophil to lymphocyte ratio seems to indicate immune competence[14].

Similarly the oncograms of the best surviving breast cancer patients (patient code starting with R, figure 2) seemed to differ from the worst surviving controls (Supplementary Fig 1). This however,

was not as evident with lung cancer (n=4, patient code starting with K) or sarcoma (n=4, patient code starting with S).

Patient by patient viral treatments, imaging responses and survival are shown in figure 3. Patients are grouped by tumor type. The individualized patient treatments and variable responses can be noted from this figure. With some patients (C332, O198, R218) imaging seemed to predict prognosis as these patients are still alive. On the other hand patient I98 responded only partly and patient S119 showed continuously stable disease in imaging, but both of these patients are still alive. Interestingly some patients show progressive disease after virus treatments (P251, S354, O205, R255) but still survived a relatively long time (>2 years). "False negatives" (lack of response in imaging) might be due to inflammatory pseudoprogression caused by the immune response generated by the virus at the tumor[32]. Although pseudoprogression is now well appreciated in the context of immunotherapy, this was not the case 10 years ago when ATAP patients were being treated[33]. In ATAP patients were monitored with traditional Response Evaluation Criteria in Solid Tumours (RECIST) or PET Response Criteria developed for monitoring traditional chemotherapy responses[32]. Also, it was not appreciated that immunotherapy can take a long time to work. In ATAP, patients typically stopped receiving further treatment if the first imaging (after a median of 63 days)[32] was not indicative of disease control. New guidelines for monitoring immunotherapeutics have recently been introduced[34].

To take full advantage of visual presentation, mean (average) oncograms of the best surviving patients were overlaid with those of the short-surviving controls (Figure 4). Sarcoma, ovarian, breast and lung cancer patients are shown by tumor type since more than three patients were among the "top 30" (Figure 4a). Interestingly, ovarian (n=7) and breast (n=4) cancer oncograms showed difference (p=0.0009 and p=0.0277 respectively) between the best and worst surviving

patients while this was not as evident for lung cancer (n=4) or sarcoma (n=4) oncograms (p=0.517 and p=0.051 respectively). The averages of the best (n=30) and worst (n=26) surviving patients' oncograms were overlaid and an area size difference was detected (p=0.000002, Figure 4b).

Despite small patient number we also looked into individual factors. With ovarian, breast and "all cancers" the difference in performance status (WHO) was significant (p=0.002, p=0.03, p<0.001 respectively). With ovarian cancer, arming of the virus was a significant component (p=0.03) while the female gender (p=0.03) showed significance in lung cancer. In addition to performance status, neutrophil to lymphocyte ratio (NLR) and neutralizing antibodies (NAb) against the treatment virus showed significant differences (p=0.001 and p=0.04 respectively) in the "all cancers" group. The leukocyte difference here was borderline (p=0.054).

Discussion

The oncogram approach was developed by combining individual factors that have been suggested to predict good survival following oncolytic virus treatment[14, 25, 28]. The manner of presentation was inspired by the immunogram approach where seven parameters were described[9]. The main difference between the oncogram and the immunogram is that the former is being proposed as a patient-by-patient practical decision-making tool for patients being considered for oncolytic adenovirus treatment while the latter is a more theoretical concept that might broadly apply to immunotherapy but has not been applied to patients yet. Important practical aspects of the oncogram include that all of the variables can be measured at baseline, and without the need for biopsies or expensive techniques. Of note, the oncogram is a patient-specific tool which considers clinical factors and also treatment specific factors such as virus arming.

Many similarities can be found between the parameters of the immunogram and the factors we found significant in our ATAP series, which were then included in the oncogram. Both recognize that blood lymphocytes and other immunological soluble markers play a role. In the immunogram, factors such as lymphocytes, Il-6, CRP, LDH are suggested based on theoretical considerations while in oncolytic virus treated patients leukocytes, neutrophil-lymphocyte ratio, IL-8 and HMGB1 were found significant following actual measurements in patients[14]. Clinical factors such as gender, performance score, tumor type or metastatic burden are not taken into account in the immunogram while tumor load and site of metastases was found relevant in oncolytic virus treated patients.

Five out of the seven variables proposed in the immunogram would need tumor biopsy which is not always practical. None of the variables present in the oncogram require biopsy and all can be measured at baseline with inexpensive widely available techniques. Also, even if biopsies were available, there are no standardized ways for measuring mutational load, interferon gamma sensitivity or glucose utilization (immunogram parameters). Even measurement of intratumoral T-cells and PD-L1 currently lack standardization despite the importance of these factors in predicting efficacy of PD-1/PD-L1 inhibitors [1-3, 12, 13].

Some of the factors captured in the oncogram are intuitive. For example, patients with good performance status (WHO) at baseline were overrepresented in the thirty long term survivors. Perhaps the immune system of such patients is better capable of mounting an anti-tumor immune response. However, good performance score patients might have lived longer even without any treatment. When ATAP was started in 2007 oncolytic viruses were thought to act mostly through oncolysis, which might be expected to occur rapidly, creating rationale for treatment of even late stage patients. However, what has been learned is that the main effect of oncolysis seems to be

induction of the anti-tumor immune response, and this can take time[6]. For example, in the oncolytic herpes T-Vec Phase 3 trial, almost half (23 out of 48) of the durable responses showed progression prior to response defined as the appearance of a new lesion or >25% increase in total tumor area. Many responses were seen only after several months or even after a year[35]. The same has been seen for anti-PD1 drugs [1-3, 12, 13]. Similarly pointing at the importance of the immune response in ATAP, long term survivors were frequently treated with viruses armed with immunostimulatory molecules (GM-CSF[11] or CD40L[27]). Nevertheless, oncolysis and tumor transduction could play a role as suggested by the finding that the longest survivors had less neutralizing antibodies against the treatment virus.

The immunogram authors proposed that the approach could help to choose the right immunological treatment between PD-1 blockade, combined PD-1 and CTLA-4 blockade or T-cell treatment. We believe oncolytic viruses could be added to the treatment arsenal as these are proposed to be potent in the "cold" non-inflamed tumor environment[36] where checkpoint inhibitors and other immunotherapeutics appear to have poor efficacy. We and others have suggested that oncolytic viruses work best in tumors with a low amount of immune cells[10, 11, 13-16]. In contrast, checkpoint inhibition works best when the tumor features neoantigens, resulting in tumor infiltrating lymphocytes and (probably reactive) PD-L1 expression [1-3, 12, 13]. Logically, excellent preliminary results have already been seen when oncolytic virus treatment was combined with checkpoint inhibition. With this combination a 62% response rate and a 33% complete response rate in advanced melanoma was noted[13]. However, although the combination appears very well tolerated, for some patients it might represent overtreatment. The oncogram, with or without the immunogram, could help identify patients for whom single or combined therapy should be used.

The oncogram presented here is designed to evaluate patients most suitable for oncolytic immunotherapy. If tumor biopsies were available it would be possible to systematically analyze many immunological factors as has been done with the Immunoscore for example[37]. Although the oncogram as presented here does not require biopsies, having tissue might improve the decision-making process further. In some individual ATAP patients biopsies before and after treatment were available. Their analyses suggested that tumors with a low amount of immune cells pre-treatment respond to oncolytic virus treatment in conjunction with recruitment of immune cells to the tumor during treatment. In contrast, tumors with an extensive immune infiltrate pre-treatment did not respond and little difference was observed in the post-treatment biopsy[11, 36]. This is in line with our proposal: Patients with large area oncograms and cold tumors (low amount of immune cells, few neoantigens, low PD-L1) could be treated with oncolytic viruses while patients with small oncograms, hot tumors with ample immune cells and high PD-L1 staining could be considered primarily for checkpoint therapies or checkpoint combinations. Intermediate and mixed cases, which probably includes most patients in a real-life situation, could be treated with the oncolytic virus + checkpoint inhibitor combination. Obviously, all of these notions require prospective evaluation in trials.

A key attraction of the oncogram is the ease of evaluating the 11 variables. Some are fairly obvious (gender, tumor type), while many others are already routinely evaluated (leukocytes, NLR, tumor load, location of metastases, performance score). The remainder (antibodies, HMGB1, IL-8) require a simple blood test followed by an inexpensive assay. In a real world situation it is unrealistic to expect that extensive tumor materials, such as needed for multiple complex analyses, would be available from patients undergoing routine treatment, especially when considering tumor types such as pancreatic cancer or glioma, or tumor recurring at distant sites with metastases. Sometimes only a fine needle biopsy can be done to confirm the diagnosis in

such cases. It is also increasingly clear that the immunological and mutational status of different metastases vary significantly, and thus a single biopsy from one tumor or metastasis might not give a comprehensive tumor-immunological view of the patient[38]. In addition to or as a replacement to biopsies it is conceivable that immunological evaluation of tumors can also be performed by imaging. This seems especially evident with magnetic imaging or magnetic spectroscopy[28]. Interesting novel approaches which could eventually complement the oncogram include non-invasive PET based T-cell imaging or PD-L1 imaging[39-41].

In conclusion, we believe that in the future cancer treatment will become more individualized, and this applies also to immunotherapy. This means that more factors, including multiple immunological markers, should be taken into account for optimizing drug selection and sequencing of treatments. The oncogram presented here constitutes a patient-data-driven hypothesis for choosing suitable patients for oncolytic therapy. More elaborate immunological analysis of tumor biopsies, blood, the lymphatic system or immunological imaging might further help to choose the optimal treatment for each patient. Clinical trials are needed to validate these preliminary findings.

Author contributions

OH, MO and AH designed and wrote the preliminary draft of the manuscript. Display items were done by OH and MO. Data management and analysis were done by OH, MO, KT, IL, AK. All authors contributed to the writing.

Acknowledgements

For biostatistical help we thank Taija Alatalo and Paula Bergman from the Biostatistics consulting, Department of Public Health, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

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Figure legends

Fig 1 Survival of all ATAP patients (2), including the subgroups of the best (1) and worst (3) survivors.

Fig 2 Oncograms of the best surviving patients. Values at the outer rim indicate good prognostic or predictive variables while values at the inner rim indicate the opposite. Data that was not available is indicated with data points in between and the outer rim does not have labeling. Good prognostic variables, as recorded before first oncolytic virus treatment, include: 1) female gender 2) WHO 0-1 3) cancers other than melanoma, colorectal, pancreatic, hepatocellular or cholangio 4) low tumor load 5) peritoneal metastases without liver metastases 6) low neutrophil to lymphocyte ratio 7) low leucocyte value 8) low IL-8 9) low HMGB1 10) First treatments with GM-CSF or CD40L armed virus 11) no anti-viral neutralizing antibodies.

Fig 3 Swimmers plot: patient by patient oncolytic virus treatments, responses and survival.

Fig 4 a) Average oncograms by tumor type of the best surviving patients (when more than three patients per group were present) compared to the worst surviving controls. b) Average oncograms of all best surviving patients (N=30) compared to the worst surviving controls (N=26). * P<0.05 ** P<0.01 *** P<0.001 Fisher´s exact test. When all variables (best survivors N=243, worst survivors N=174) were compared P=0.000002.

Table 1 Patient features before first oncolytic virus treatment

Table 2 Patient treatments, responses and survival

Suppl Fig 1 Oncograms of the worst surviving patients. Values at the outer rim indicate good prognostic or predictive variables while values at the inner rim indicate the opposite. Data that

was not available is indicated with data points in between and the outer rim does not have labeling.

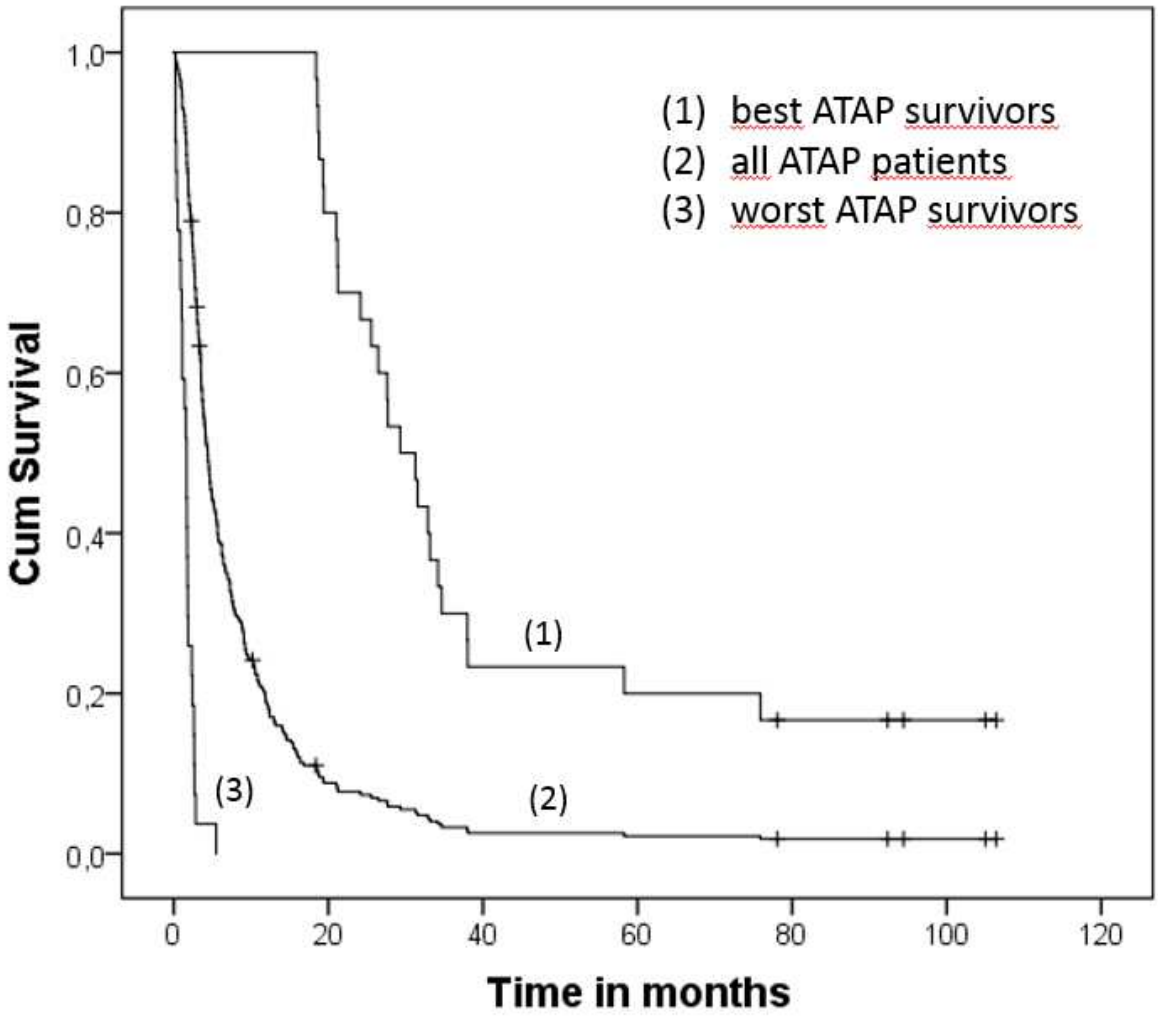
Suppl Table 1 Individual patient features and prior treatments

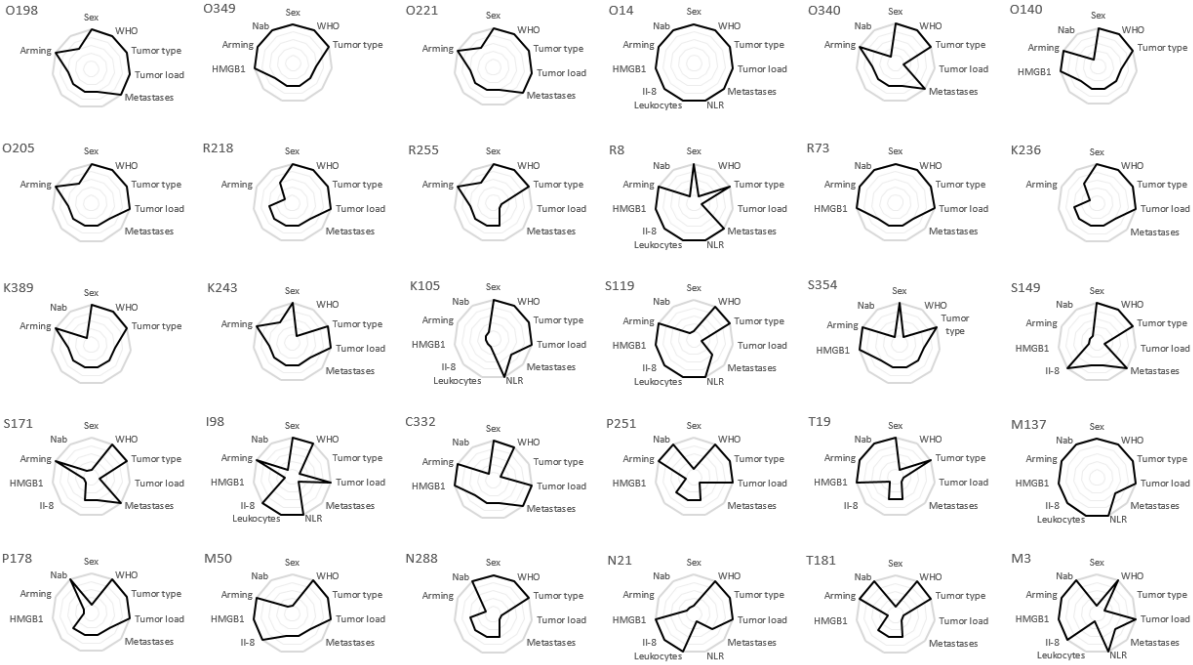
Supplementary table 2

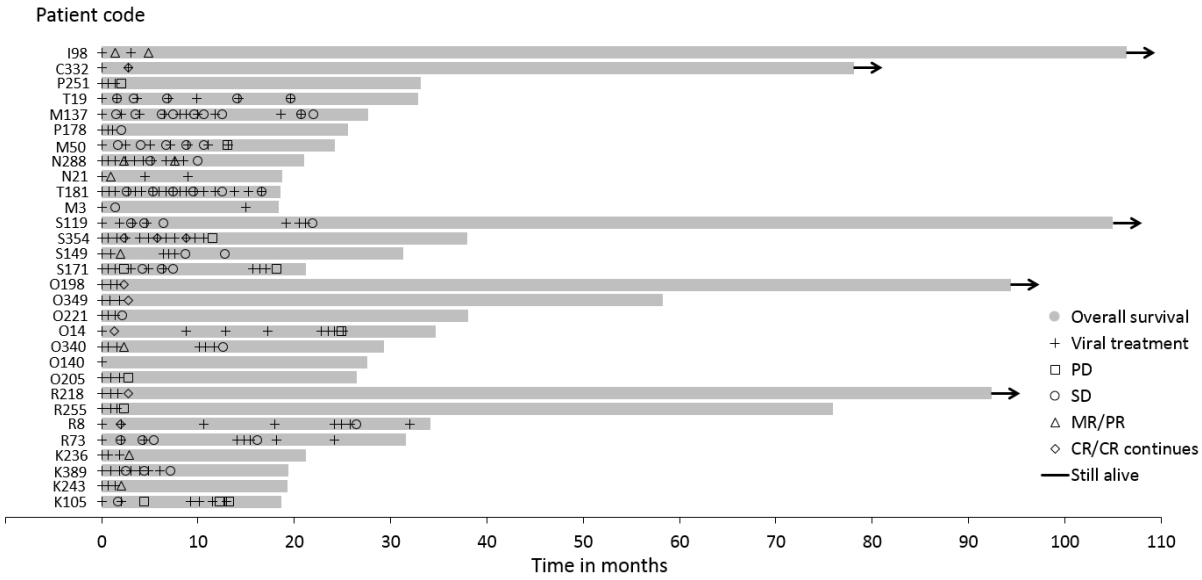
Significant data for Overall survival/Cancer mortality			**Ref**
Variable (suggesting good OS, outer ring in the oncograms)	**P value**	**HR (95% CI)**	
Sex (female)	**0.017** **0.043**	**0.694 (0.514–0.936)** **0.77 (0.59-0.99)**	**14** **18**
WHO (0-1)	**<0.001** **<0.001** **0.010** **0.002**	**0.410 (0.314–0.535)** **0.108 (0.048–0.244) WHO 0** **0.163 (0.081–0.328) WHO 1** **0.24 (0.08-0.71)** **0.467 (0.288-0.755)**	**14** **27** **18** **29**
Tumor type (cancers other than melanoma, colorectal, pancreatic, hepatocellular or cholangio carcinoma**)**	**0.038 Lung** **0.006 Gynecological** **0.001 Other** **0.009 Urogenital** **0.004 Head and Neck** **0.0003 Ovarian**	**0.535 (0.296–0.966)** **0.509 (0.316–0.821)** **0.501 (0.329–0.762)** **0.412 (0.211–0.805)** **0.10 (0.02-0.47)** **0.08 (0.02-0.29)**	**14** **27** **18**
Tumor load (lower than median)	**0.003**		**14**
Metastases[1)] (presence of peritoneal metastases, no	0.065 **(0.021 RR) no liver** 0.272 (0.094 RR) peritoneal		**14**

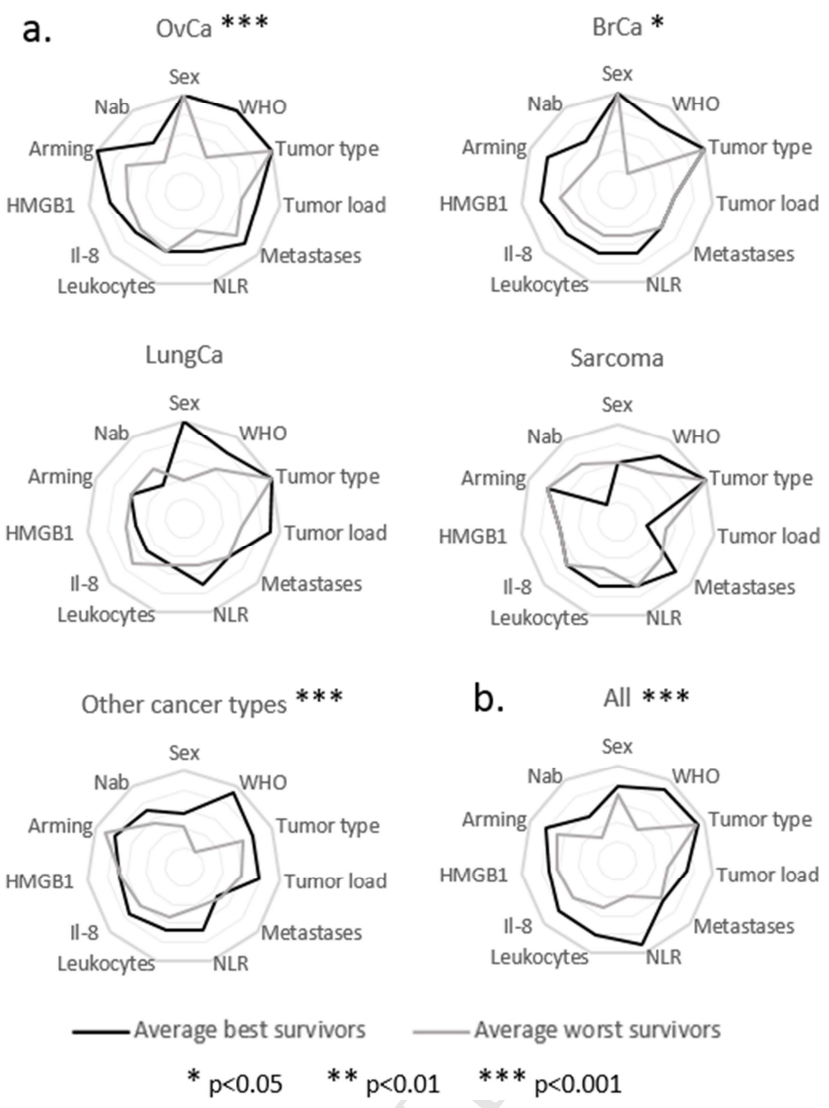
liver metastases)			
NLR (lower than median)	**< 0.001**	**3.003 (1.896-4.755)**	**14**
Leucocytes (lower than median)	**0.008**		**14**
Il-8 (<62ng/l="normal" value)	**0.010**	**0.502 (0.297-0.847)**	**29**
HMGB1 (<512ng/l, lower than median)	**0.006**	**0.462–0.881**	**27**
Arming (treatment with CD40L or GM-CSF producing virus)	**0.006 CD40L** **<0.001 GM-CSF**	**0.439 (0.245–0.788)** **0.378 (0.228–0.628)**	**14**
NAb (no NAbs against adenovirus)	**0.033**	**0.689 (0.490–0.970)**	**14**

HR hazard ration, CI confidence interval, RR imaging response rate, NLR Neutrophil-to-lymphocyte ratio, Il-8 Interleukin 8, HMGB1 High mobility group box 1, GM-CSF Granulocyte-macrophage colony-stimulating factor, NAb neutralizing antibody
[1)] Significance was found only in RR, however we decided to combine the metastasis data in the oncograms as suggestive trends were also seen in OS and presence on peritoneal metastasis seemed to suggest good RR and OS after virus treatments


(1) best ATAP survivors
(2) all ATAP patients
(3) worst ATAP survivors
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
Time in months
(1)
(2)
(3)







* p<0.05 ** p<0.01 *** p<0.001